CHAPTER - 2

SCOPE AND SUMMARY
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Since the work of Disenhoffer et al. on the photosynthetic apparatus of a bacterium in 1985, the work on distant effect has been carried out vigorously. The phenomenon of distant effect has been examined by many workers by a space effect or by using a spacer. In the later case, the covalent bond distance in the spacer gives an idea as to the extent of distance between two reactive sites.

The elucidation of the mechanism of the hydrolysis of schiff bases has been a subject of interest in our laboratory. In the present work schiff bases with spacers of different length have been derived so that the influence of spacers can be examined in the hydrolysis.

The Chapter-3 of the thesis deals with the synthesis of schiff bases (I) with \( n = 2, 3, 4 \) and 6.
The synthesised and crystallised Schiff bases are characterised by spectral and elemental analysis. These compounds are found to be insoluble in water but go into solution in 2% dioxane-water (v/v) system. Therefore, the spectra of the Schiff bases in various aquo-organic solvent systems have been studied and the results are reported in this Chapter. It is interesting to note that with increasing content of the organic component in the solvent system the peaks at around 398 nm and 275 nm due to $\pi - \pi^*$ and $n - \pi^*$ transitions gradually decrease in intensity with the appearance of peaks at 318 and 255 nm respectively. The isosbestic points are independent of the nature of the organic solvent. It has been assumed, therefore, that the Schiff bases need a minimum amount of organic solvent to develop the required topology to go into solution in water or in the organic solvent. The compounds therefore appear to be distributed in the solvent mixture. The oil coated solute get solubilised in organic solvent ($C_o$) and in water environment ($C_w$). The concentration of both the species have been calculated and the ratio has been found to increase with increasing amount of organic solvent in the mixture. The points of intersection are found to be invariant and occur at 70% by volume of the organic component. Further, the volume percents of the organic solvent at which $C_o = C_w$ ($\nu_{C_o=C_w}$) are found to increase with increasing spacer and a
plot of $V_{c_{o}} - V_{c_{w}}$ vs. $n$ becomes linear asymptoting after $n = 4$.

These observations have lead us to propose the presence of intramolecular hydrogen bonded structure (II) and the zwitterionic structure (III) in an equilibrium for all the
The zwitterionic structure is in resonance with an orthoquinonoid structure (IV). Presence of slight amount of water changes the equilibrium towards III and therefore water plays a vital role in the formation of zwitterionic structure. It appears to be filling up the space between two phenoxy groups (Fig. I).

Fig. I: Schiff bases with hydrophillic pocket filled with water.

The kinetics of hydrolysis of the schiff bases at 2% dioxane-water (v/v) has been carried out by varying the pH of the solution. The products of the hydrolysis have been found to be salicylaldehyde and the diamine. The formation of the products have been confirmed by electronic and NMR spectra. It is interesting that decreasing the pH of the solution does not generate a hypsochromic effect due to
protonation at the phenoxy anion but increases the rate of the reaction.

The logarithm of the first order rate constants \( \log k_\psi \) vs. pH are found to be linear with a sensitivity index \( m \) (equation - i). The sensitivity index \( m \) and the spacer length \( n \) vary linearly with a negative slope and

\[
\log k_\psi = \log k_{\psi,0} - m \times pH \quad \ldots \,(i)
\]

\( m = 0 \) when \( n = 11 \). The inability to protonate the phenoxy anion has been ascribed to the capture of the proton by the water pool in the intervening space. The pKa of the schiff bases have been determined and the values are within 3.9 - 4.6. A linear free energy relationship (equation - ii) has been obtained to show the effect of spacer on pKa values.

\[
(pK_a)_n = (pK_a)_0 - 0.18n \quad \ldots \,(ii)
\]

The first order rate constants are found to decrease with increase in spacer chain at all pH. The decrease is precipitous when \( n \) changes from 2 to 3 and then becomes gradual to \( n = 6 \). The dependence on spacer on the rate constant is attributed to the intramolecular catalysis (Fig.II) by both the phenolate ions. With increasing spacer
the possibility of the intramolecular catalysis decreases as a result the rate also decreases. It is also interesting to observe that the 395 nm peak gradually shifts to 378 nm with decreasing optical density. The 378 nm peak is due to \( \text{SA}^- \) (V) or \( \text{SB}^- \) (VI). The appearance of the 378 nm peak in this work has been ascribed to \( \text{SA}^- \) and not \( \text{SB}^- \). Further, salicylaldehyde (SA) does not give \( \text{SA}^- \) in dioxane-water.

\[ \text{V} \quad \text{VI} \]
Therefore, it has been proposed that the Schiff bases undergo hydrolysis to $SA$ and $SA^-$ by independent mechanisms (Scheme - i). With increasing dioxane content in the solvent mixture the rate constant remains same up to 40%dioxane (v/v) and then decrease to a negligible value in 80% by volume of dioxane. Further the shift of 398nm peak to 378nm peak also decreases sharply with increasing dioxane content. It has been argued that with decreasing water content, the extent of intermolecular attack of water decreases resulting in the formation of lesser quantity of $SA^-$. 

The rate of hydrolysis has also been studied by NMR technique by following the increasing intensity of the aldehyde proton peak at 10.2 $\delta$ and the decreasing intensity of the aldimine proton peak at 8.6 $\delta$. Both these methods give almost same value of the rate constants and are of the same order as determined by electronic spectra.

The hydrolysis has also been carried out in presence of metal ions like Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$, Both Ni$^{2+}$ and Zn$^{2+}$ ions do not produce a hypsochromic shift in the spectrum of the Schiff bases although salicylidene ethylene diamine and salicylidene methylene diamine produces a hypsochromic shift of 48nm on addition of Zn$^{2+}$. This difference in behaviour may be ascribed to the spacer in the compounds under investigation. Both these metal ions enhance
Scheme i

A:

\[ \text{R} \text{CH}_2 \text{N}^+ = \text{O}^\ominus \text{H} \overset{\text{H}_2\text{O}}{\longrightarrow} \text{R} \text{CH}_2 \text{N}^+ \text{H}_2\text{O}^\ominus \]

\[ \text{R} \text{CH} + \text{H}_2\text{N}-\text{R} \underset{\text{H}_2\text{O}}{\rightleftharpoons} \]

\[ \text{R} \text{CH} = \text{O}^\ominus \text{H} \overset{\text{H}_2\text{O}}{\longrightarrow} \text{R} \text{CH} \text{N}^+ \text{H}_2\text{O}^\ominus \]

B:

\[ \text{R} \text{CH}_2 \text{N}^+ = \text{O}^\ominus \text{H} \overset{\text{H}_2\text{O}}{\longrightarrow} \text{R} \text{CH}_2 \text{N}^+ \text{H}_2\text{O}^\ominus \]

\[ \text{R} \text{CH} + \text{H}_2\text{N}-\text{R} \underset{\text{H}_2\text{O}}{\rightleftharpoons} \]

\[ \text{R} \text{CH} = \text{O}^\ominus \text{H} \overset{\text{H}_2\text{O}}{\longrightarrow} \text{R} \text{CH} \text{N}^+ \text{H}_2\text{O}^\ominus \]

\[ \text{R} \text{CH} \text{OH} + \text{R}-\text{NH}_2 \]

\[ \text{R} \text{CHO} \]

Scheme i
the rate of hydrolysis but their influence decreases sharply from \( n = 2 \) to \( n = 3 \) and gradually to \( n = 6 \) with increasing spacer in the bases. The \( \text{Cu}^{2+} \) ion, however, behaves differently. When \([\text{Cu}^{2+}] < [\text{SB}]\), the spectrum continues to show a peak at 398 nm but when \([\text{Cu}^{2+}] > [\text{SB}]\), a peak at 346 nm appears with complete absence of 398 nm peak which may be due to its binding with the phenoxy anion. After this binding, the hydrolysis becomes extremely slow. This work therefore, gives an idea that the enzymatic hydrolysis of schiff base leading to a biochemically favourable reaction is facilitated by \( \text{Zn}^{2+} \) and \( \text{Ni}^{2+} \) ions whereas \( \text{Cu}^{2+} \) ion acts as a poison.

Since increasing spacer in schiff bases results in greater hydrophobicity of the molecules the hydrolysis of the compounds has been studied in presence of surfactants like NaLS, TX-100 and CTAB. The compounds do not produce any change in spectrum in CTAB whereas in NaLS and TX-100 the spectra are interesting. In both the surfactants (NaLS and TX-100), the schiff base generate spectrum like those in aquo-organic solvent mixture indicating the existence of both schiff base within the micelle and \( \text{SB}(\pm) \) outside the micelle in equilibrium. The isosbestic points in NaLS are spacer dependent which may be due to the different interaction of the schiff bases with micelles. The plots of optical density at 398 nm or 318 nm vs [surfactant]
give intersecting linear plots from which the critical micellar concentration (CMC) values have been evaluated. These values agree with the literature value and are spacer dependent in NaLS. In TX-100, however, the values remain constant. The binding constants have also been evaluated by following the equation (iii).

\[ K[S] = \frac{A - A_o}{A - A_\infty} \quad \ldots (iii) \]

In this case also, two intersecting straight lines are obtained which give the CMC values of the surfactants. The slopes of the linear plots have yielded the values of binding constants \((K_1)\) and \((K_2)\) with surfactants before and after CMC. The \(K_2\) values are found to change with increasing spacer length in NaLS micelle and a slope of \(5.3 \text{ M}^{-1}\) is obtained. This is to say that association constant increases by 5.3 per methylene group which is in conformity with the literature value.

The time dependent electronic spectra of the schiff bases in surfactants also show interesting results. When the [surfactants] > CMC, the initially observed 398nm peak changes to 379 nm peak with time and then the optical density of the 378 nm peak increases with time. After reaching a constant optical density value 378nm peak depletes, the rate of which is much slower in NaLS micelles.
than in TX100 micelles. Therefore, the overall rate of hydrolysis has been followed at 420 nm peak. The rate constants do not change substantially in various concentrations of the surfactants. The spectral behaviour have been explained by a micellar catalyzed proton transfer process (Fig. III).

![Micellar catalysed proton transfer mechanism.](image)

Fig. III: Micellar catalysed proton transfer mechanism.
The following conclusions have been made from this work:

(i) The presence of spacer makes the compound flexible like enzymes so that the topology of the molecules changes with the environment in a non-fluggy manner.

(ii) The spacer incorporates water molecules within the intervening space and the water molecules behave as a transport medium for $H^+$ and metal ions like $Zn^{2+}$ and $Ni^{2+}$.

(iii) The NaLS micelles like membranes accept and release protons depending on the nature of the substrate.